

Effects of Between-Row and Within-Row Spacing on Alfalfa Seed Yields

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ABSTRACT

Proper between-row and within-row spacing is essential for optimizing alfalfa seed yields and stand longevity. Using three alfalfa (*Medicago sativa* L.) cultivars (WL232HQ, Derby, and Algonquin), we conducted a field study from 2004 to 2007 to evaluate the effects of three between-row spacing treatments (60, 80, and 100 cm) and four within-row spacing treatments (15, 30, 45, and 60 cm) on seed yield, seed yield components, plant height, basal stem diameter, and lodging. Our hypothesis was that the intermediate between-row and within-row spacing would gradually improve seed yields in later years. The highest seed yields were obtained with 60-cm between-row spacing and 15-cm within-row spacing in 2004, but with 80-cm between-row spacing and 30-cm within-row spacing in 2006 and 2007. The significant year \times between-row spacing and year \times within-row spacing interactions for seed yield indicated that 80-cm between-row spacing and 30-cm within-row spacing produced the best seed yields as years advanced, and our results confirmed this. With the increase of within-row spacing, stems per square meter decreased, while racemes per stem increased significantly. The effects of between-row and within-row spacing on seeds per pod, however, were not significant in four years. The results suggest that 80-cm between-row spacing and 30-cm within-row spacing can decrease the risk of lodging and optimize seed yields in the third and fourth harvest years.

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ALFALFA (*Medicago sativa* L.) is a forage crop that is grown worldwide, but its seed yield is considered to be of secondary importance (Iannucci et al., 2002). However, seed yield of alfalfa is important in determining the effective distribution of new cultivars to farmers (Bolanos-Aguilar et al., 2002). Successful seed production of alfalfa requires special climatic conditions (Abu-Shakra et al., 1969). In the semiarid cropping region of northwestern China, the climate is characterized by low humidity and moderate air temperature, which are suitable for alfalfa seed set, pollinator activity, and low incidence of disease (Grandfield, 1945; Rincker et al., 1988). Additionally, the mountain run-off water and the groundwater aquifers in this region provide ample amounts of irrigation water. It is predicted that this region has the potential for specialized alfalfa seed production, supplying both the domestic needs for China, as well as, the export markets.

Many factors including environment, genotype, and agronomic techniques, influence alfalfa seed yield and seed quality (Dordas, 2006). Genetic diversity for alfalfa seed yield, seed yield components, and the traits influencing seed yield have been described (Knapp and Teuber, 1994; Rosellini et al., 1998; Bolanos-Aguilar et al., 2000). However, the actual seed yield improvement

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achieved in breeding has been limited (Bolanos-Aguilar et al., 2002), and alfalfa breeding programs still focus on improving the yield, quality, and resistance to insects, disease, and abiotic stresses. Thus, it is important to determine the appropriate agronomic factors that optimize both seed yield and seed quality (Hampton, 1991).

Establishing and maintaining a specific plant density in an alfalfa stand can best be achieved by using the optimum between-row and within-row spacing when planting the field. Many studies have been conducted on the effects of thinning and between-row spacing on alfalfa seed yield at multiple locations. According to Pederson (1957, 1962), plants in thinned stands of alfalfa were several inches shorter, lodged less, and were more accessible to pollinating insects than those in unthinned stands. Recommended between-row spacings in these studies were quite different and varied from 20 to 91 cm (Abu-Shakra et al., 1969; Dovrat et al., 1969; Rincker, 1976; Kephart et al., 1992; Askarian et al., 1995; Kowithayakorn and Hill, 1982). Only a few previous studies referred to the effect of within-row spacing on seed yield (Rincker, 1976; Kowithayakorn and Hill, 1982). Seed yield is the product of a number of individual yield components. The effects of plant density were significant on stems per square meter and racemes per stem, but not consistent on pods per raceme and seeds per pod (Abu-Shakra et al., 1969; Kowithayakorn and Hill, 1982; Askarian et al., 1995). Furthermore, the effects of between-row spacing and within-row spacing can vary with years, thus influencing several years' production and stand longevity.

This field research was designed to determine the optimum between-row and within-row spacing for successful seed production of alfalfa, with special emphasis on yearly changes comparing seed yields under three between-row and four within-row spacing treatments. Our hypothesis was that the intermediate between-row and within-row spacing would enhance one or more alfalfa yield components, gradually improving seed yields in latter years. The second objective of this study was to test the effects of between-row and within-row spacing on plant height and basal stem diameter and their correlation with lodging.

MATERIALS AND METHODS

Research Location and Experimental Design

The field experiment was conducted at the China Agricultural University Grassland Research Station located at the Hexi Corridor, northwestern China (latitude 39°37' N, longitude 98°30' E; elevation 1480 m) from 2004 to 2007. Soil at the site is Mot-Cal-Orthic Aridisols, classified as Xeric Haplocalcids in the USDA soil classification (Soil Survey Staff, 1996). Initial chemical characteristics of the soil (0–30 cm) were pH 8.4, organic matter 8.15 g kg⁻¹ dry matter, total N 0.609 g kg⁻¹, available P 11.0 mg kg⁻¹ (Olsen method), and available K 143.5 mg kg⁻¹ (NH₄Ac). Soil pH was measured using a 1:2 soil-to-water ratio (Watson and Brown, 1998). Organic matter of soil was estimated using the modified Walkley–Black method of Nelson and Sommers (1982). Total Kjeldahl nitrogen was determined using the standard digestion of Issac and Johnson (1976). Available P was determined by sodium bicarbonate (NaHCO₃) extraction and subsequent colorimetric analysis (Olsen et al., 1954). Available K was determined using an ammonium acetate extraction followed by emission spectrometry (Knudsen et al., 1982). Three weather variables (precipitation, average temperature, and occurrence of gales) during the growing seasons are reported as mean monthly data in Table 1. A gale is defined as a wind with speeds of 17.2 to 20.7 m s⁻¹, according to the Beaufort scale. The previous crop, before study establishment, was tall fescue (*Festuca arundinacea* Schreb.). The grass stand was plowed and was fallow for one year before establishing the alfalfa study.

The experiment used a randomized complete block design with four replications. Each replication had 36 treatment combinations. Treatments were arranged as 3 × 4 × 3 factorial combination of three between-row spacings (R) (60, 80, and 100 cm), four within-row spacings (I) (15, 30, 45, and 60 cm), and three alfalfa cultivars (WL232HQ, Derby, and Algonquin). Individual plot size was 4.5 m by 8 m, with 1.5-m spacing between the adjacent plots.

The sowing seed of three cultivars (WL232HQ, Derby, and Algonquin) was provided by W-L Research, Inc. (Madison, WI), Barenbrug Holland B.V. (Nijmegen, Netherlands), and SW Newfield Seeds Company, Ltd. (Nipawin, Canada), respectively. These cultivars were chosen for evaluation on the grounds of their high adaptability and widespread use in the region. The trial was established on 15 July 2003. Hole-seeding was used, in which 8 to 10 seeds per clump were planted at the depth of 1 to 2 cm. Clump density (clumps per square

Table 1. Precipitation, average air temperature, and occurrence of gales between March and August for 2004, 2005, 2006, and 2007 at the research location.

Month	Precipitation				Avg. temp.				No. of gales [†]			
	2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007
	mm				°C							
Mar.	4.7	28	6.1	6.3	4.1	2.1	2.5	2.2	4	0	0	1
Apr.	0.7	1.5	1.8	20.6	13.3	11.6	11.7	10.0	1	3	6	1
May	6.2	8.6	9.6	4.0	16.2	17.0	16.2	15.5	1	0	3	0
Jun.	18.1	4.1	2.0	7.1	20.1	22.3	21.6	21.0	0	2	2	1
Jul.	10.2	3.6	52.4	40.8	22.7	23.2	22.0	22.0	0	2	2	1
Aug.	13.2	9.8	3.8	19.9	20.0	20.7	21.5	21.4	1	0	0	0

[†]Gale = wind with speeds of 17.2 to 20.7 m s⁻¹, according to the Beaufort scale.

Table 2. The clump densities calculated from the 36 combination of between-row spacing, within-row spacing and cultivar treatments.

Combination of between-row and within-row spacing		Clumps density of three cultivars
Between-row	Within-row	
cm		clumps m ⁻²
60	15	11.11
60	30	5.56
60	45	3.70
60	60	2.78
80	15	8.33
80	30	4.17
80	45	2.78
80	60	2.08
100	15	6.67
100	30	3.33
100	45	2.22
100	60	1.67

Table 3. Dates of flowering and harvesting in 2004, 2005, 2006, and 2007.

	Year			
	2004	2005	2006	2007
Flowering date	14 June	11 June	15 June	12 June
Harvesting date	5 Aug.	2 Aug.	7 Aug.	4 Aug.

meter) with each of the combinations of three between-row spacing and four within-row spacing treatments is shown in Table 2. Seeds were inoculated with a commercial inoculant of *Sinorhizobium*. Initial fertilizer was applied as diammonium phosphate [(NH₄)₂HPO₄] at a recommended rate of 225 kg ha⁻¹. The exact amounts of the N and P applied in the first year were 47.7 and 52.8 kg ha⁻¹, respectively. The purpose of adding N fertilizer was to accelerate the growth of alfalfa seedling, improve frost resistance, and ensure the successful establishment of experimental field. Following the July seeding, irrigations (90 mm each application) were provided on 18 July, 27 July, and 26 November, respectively, for a total of 270 mm of supplemental water in 2003. During the green-up period in 2004, each clump was hand-thinned to three plants per clump. In each subsequent year, superphosphate was banded at the rate of 100 kg ha⁻¹ of P₂O₅ in early March, 5 cm from one side of each row and 5 cm deep. The experimental field trial was irrigated in mid-May and November every year at a rate of 90 mm of supplemental water per application. Thus, the average amount of supplemental water applied each year after the establishment year was 180 mm. Weeds were controlled with hand hoeing as needed throughout the growing seasons. Pollination during the seed production years was provided primarily by honeybees (*Apis mellifera* L.), although other pollinators such as bumblebees (*Bombus* spp.) were observed in low populations.

Data Collection

Actual seed yields were determined by hand harvesting eight random clumps from each plot when 75% of the pods turned blackish brown. At the time of harvest, seed moisture content was approximately 17%. The harvesting dates of each year are provided in Table 3. The seed samples of each plot were dried, threshed cleaned,

sieved, and weighed and then stored in paper bags before laboratory testing. Seed yield was calculated with seed at 13% standard moisture content (kg ha⁻¹).

The five seed yield components considered included stems per square meter, racemes per stem, pods per raceme, seeds per pod, and 1000-seed weight (g). Before seed harvest, six random clumps were sampled from each plot to determine the numbers of stems per clump. The number of stems per square meter was calculated by multiplying the average number of stems per clump by clump density (Table 2). Thirty stems, 60 racemes, and 60 pods were randomly sampled from each plot to determine the average numbers of racemes per stem, pods per raceme, and seeds per pod. Seeds per pod data is not shown because differences in seeds per pod were not significant in different treatments over the four years of the study. The 1000-seed weight was determined using four random samples of clean seeds from each plot, which had been dried at 80°C to constant moisture content.

Plant height and basal stem diameter were determined by taking measurements on 30 stems selected randomly in each plot. Flowering dates were recorded when 50% of the stems had at least one flowering inflorescence (Table 3). Lodging was evaluated visually for each plot during the flowering period, using a scale of 1 to 5, where 1 denotes no lodging and 5 denotes when the plants are 100% lodged. Because very little lodging occurred in 2004, plant height, basal stem diameter, and lodging status were not recorded.

Statistical Analysis

The experiment was conducted for four consecutive years (2004, 2005, 2006, and 2007) in one location. Years were treated as a fixed effect because the experiment was designed to test what would occur as the alfalfa stands matured. Years, three treatments effects (between-row spacing, within-row spacing, cultivars), and their interactions were analyzed using a standard *F* test. Data from each year were also analyzed separately to determine the among-year variations. Mean separations for between-row spacing, within-row spacing, and cultivar were performed using Fisher's protected LSD test at a *P* ≤ 0.05 significance level. The relationships between lodging status and two morphologic traits (plant height and basal stem diameter) were determined by correlation analysis across between-row spacing and within-row spacing treatments (*n* = 48). These analysis procedures were performed using the SPSS software (SPSS, 2000).

RESULTS

Statistical probabilities of the *F* test for year, between-row spacing, within-row spacing, cultivar, and their interactions for seed yield and yield components are summarized in Table 4. There were significant year × between-row spacing and year × within-row spacing interactions for seed yield, indicating the variable response to between-row spacing and within-row spacing effects among years.

Table 4. Statistical probabilities of *F* test for years, main effects, and their interactions on seed yield (actual seed yield and seed yield/clump) and yield components (stems/m², racemes/stem, pods/raceme, seeds/pod, and 1000-seed weight).

Source	df	Seed yield		Yield components				
		Actual seed yield	Seed yield/clump	Stems/m ²	Racemes/stem	Pods/raceme	Seeds/pod	1000-seed weight
Year (Y)	3	**	**	NS [†]	**	**	**	**
Between-row (R)	2	**	**	**	**	NS	NS	NS
Within-row (I)	3	**	**	**	**	NS	NS	**
Cultivars	2	**	*	NS	NS	NS	NS	**
Y × R	6	**	**	*	**	NS	NS	NS
Y × I	9	**	NS	*	NS	NS	NS	*
R × I	6	NS	**	**	NS	NS	NS	NS
Y × R × I	18	**	NS	**	NS	NS	NS	NS

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

†NS, not significant.

Weather Effects

During the four years of the study, the climatic conditions, especially during anthesis and seed set (June and July), were quite variable, resulting in significant yield differences among years (Table 1). The year 2004 was the best year for alfalfa seed production, whereas the unfavorable climatic conditions during anthesis and seed set periods could explain the lower seed yields obtained in 2005, 2006, and 2007. The weather in July of 2006 and 2007 was characterized by much more precipitation (52.4 and 40.8 mm) compared with average precipitation (20.1 mm). The excessive precipitation was probably detrimental to pollination and seed set and also led to excessive vegetative growth at the expense of seed production. In 2005 four gales (instantaneous wind speed >17 m s⁻¹) occurred during the summer (June, July, and August) and led to significant lodging, negatively impacting seed yields.

Seed Yield

Optimum seed yields of each cultivar varied by treatment in each of the four years. In 2004 the highest mean seed yields were obtained with 60-cm between-row spacing and 15-cm within-row spacing treatment, whereas the maximum yields were obtained with 80-cm between-row spacing and 30-cm within-row spacing in 2006 and 2007. During 2005 the mean seed yields of WL232HQ and Derby did not differ significantly among three between-row spacing and the four within-row spacing treatments (Fig. 1). The significant year × between-row spacing and year × within-row spacing interactions for seed yield verified the advantage of using 80-cm between-row spacing and 30-cm within-row spacing over all other spacing treatments for maintaining seed productivity in the third and fourth harvest years (Table 4; Fig. 1). Furthermore, the combination of 80-cm between-row and 30-cm within-row spacing resulted in the highest seed yields in 2006 and 2007 for all three cultivars (Table 5).

Table 5. Average values for actual seed yield in three cultivars under three between-row and four within-row spacing treatments in 2004, 2005, 2006, and 2007.

Between-row (R)	Within-row (I)	WL232HQ				Derby				Algonquin			
		2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007
cm	cm	kg ha ⁻¹											
60	15 [†]	1850	822	637	802	2307	589	668	700	1342	652	609	669
	30	1258	832	842	872	1079	606	749	815	929	663	732	783
	45	1109	695	817	761	1324	581	674	688	962	712	711	710
	60	906	678	802	801	850	584	618	684	973	626	626	720
80	15	1300	795	912	915	1319	640	746	871	1125	708	766	892
	30	1070	779	942	1064	1059	652	788	946	1147	693	786	1007
	45	909	771	809	913	1026	641	756	835	932	641	699	854
	60	689	768	762	737	780	649	717	705	809	647	675	697
100	15	1227	705	817	875	1027	683	715	722	1334	749	629	719
	30	1097	662	797	928	1071	658	743	781	1035	640	677	816
	45	828	679	708	688	1006	626	589	629	898	654	627	681
	60	770	613	543	563	647	537	534	611	841	568	598	606
LSD _{0.05} (R × I)		491	168	110	138	356	110	93	151	297	106	85	144

[†]Data for every treatment combination of three between-row and four within-row spacing treatments.

The mean seed yields per clump of three cultivars increased significantly with increases in between-row spacing and within-row spacing over the 4-yr period, except that in 2004 the highest mean seed yield per clump of Derby was obtained from 45-cm within-row spacing treatment. In addition, the combination of 60-cm between-row and 15-cm within-row spacing resulted in the lowest seed yields per clump in all three cultivars from 2005 to 2007 (Table 6).

Seed Yield Components

The year effect was significant at $P < 0.01$ for four seed yield components (not for stems per square meter), which partly explains the fluctuating seed yields over four years (Table 4).

Both between-row and within-row spacing treatments significantly affected stems per square meter, which decreased with an increase in between-row and within-row spacing (Table 7). Year \times within-row spacing interaction for stems per square meter showed that from 2004 to 2007, the

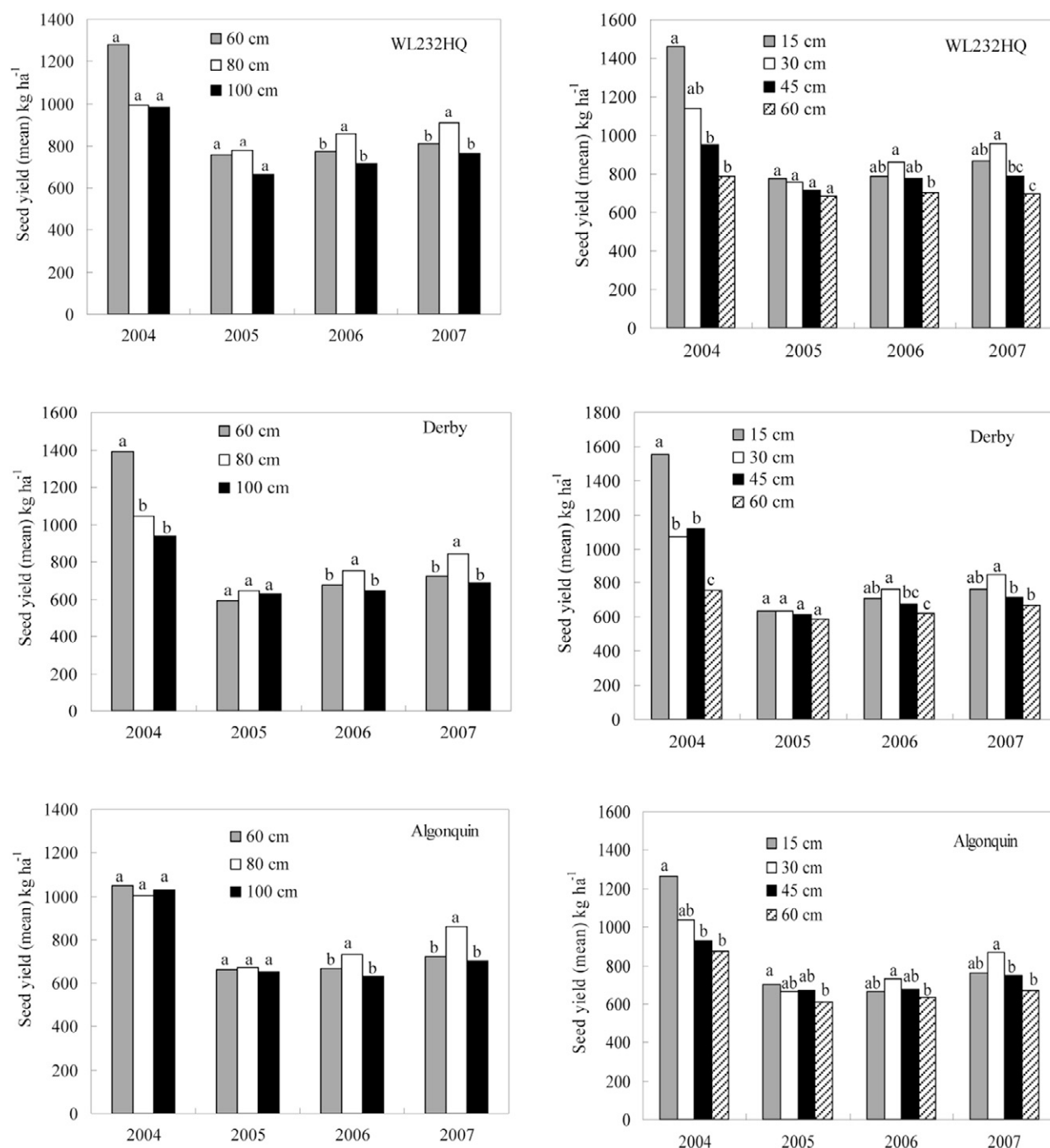


Figure 1. Interactions of year \times between-row spacing (left-hand column) and year \times within-row spacing (right-hand column) for seed yields in three cultivars (WL232HQ, Derby, and Algonquin). Data for between-row spacing treatments are pooled across within-row spacing treatments, and data for within-row spacing treatments are pooled across between-row spacing treatments under each cultivar. Means represented by bars with different letters in each graph and each year are significantly different at $P \leq 0.05$.

Table 6. Average values for seed yield per clump in three cultivars under three between-row and four within-row spacing treatments in 2004, 2005, 2006, and 2007.[†]

Between-row (R)	Within-row (I)	WL232HQ				Derby				Algonquin			
		2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007
cm	cm	g											
60	15 [‡]	16.6	7.4	5.7	7.2	20.8	5.3	6.0	6.3	12.1	5.9	5.5	6.0
	30	22.6	15.0	15.2	15.7	19.4	10.9	13.5	14.7	16.7	11.9	13.2	14.1
	45	29.9	18.8	22.1	20.6	35.7	15.7	18.2	18.6	26.0	19.2	19.2	19.2
	60	32.6	24.4	28.9	28.8	30.6	21.0	22.2	24.6	35.0	22.5	22.5	25.9
80	15	15.6	9.5	10.9	11.0	15.8	7.7	9.0	10.4	13.5	8.5	9.2	10.7
	30	25.7	18.7	22.6	25.5	25.4	15.7	18.9	22.7	27.5	16.6	18.9	24.2
	45	32.7	27.8	29.1	32.9	36.9	23.1	27.2	30.1	33.6	23.1	25.2	30.7
	60	33.1	36.9	36.6	35.4	37.5	31.2	34.4	33.8	38.8	31.1	32.4	33.5
100	15	18.4	10.6	12.3	13.1	15.4	10.2	10.7	10.8	20.0	11.2	9.4	10.8
	30	32.9	19.9	23.9	27.8	32.1	19.7	22.3	23.4	31.1	19.2	20.3	24.5
	45	37.3	30.5	31.9	31.0	45.3	28.2	26.5	28.3	40.4	29.4	28.2	30.6
	60	46.2	36.8	32.6	33.8	38.8	32.2	32.0	36.7	50.4	34.1	35.9	36.4
LSD _{0.05} (R * I)		10.6	5.8	2.9	4.3	7.6	3.2	3.5	4.7	6.5	3.0	2.5	5.1
	60 (mean) [§]	25.5b	16.4b	18.0b	18.1b	26.6b	13.2c	15.0b	16.0b	22.4c	14.9c	15.1c	16.3b
	80 (mean)	26.8ab	23.2a	24.8a	26.2a	28.9ab	19.4b	22.4a	24.3a	28.4b	19.8b	21.4b	24.8a
	100 (mean)	33.7a	24.4a	25.2a	26.4a	32.9a	22.6a	22.9a	24.8a	35.5a	23.5a	23.5a	25.6a
	LSD _{0.05} (R)	7.5	4.1	2.1	3.0	5.4	2.3	2.5	3.3	4.6	2.1	1.8	3.6
	15 (mean) [¶]	16.9c	9.2d	9.6d	10.4d	17.3c	7.7d	8.6d	9.2d	15.2d	8.5d	8.0d	9.2d
	30 (mean)	27.1b	17.8c	20.6c	23.0c	25.6b	15.4c	18.2c	20.3c	25.1c	15.9c	17.4c	20.9c
	45 (mean)	33.3ab	25.7b	27.7b	28.1b	39.3a	22.3b	24.0b	25.6b	33.3b	23.9b	24.2b	26.9b
	60 (mean)	37.3a	32.7a	32.7a	32.7a	35.6a	28.1a	29.6a	31.7a	41.4a	29.2a	30.3a	31.9a
	LSD _{0.05} (I)	8.7	4.8	2.4	3.5	6.2	2.6	2.9	3.9	5.3	2.4	2.1	4.2

[†]Means in the same column with different letters are significantly different ($P \leq 0.05$).

[‡]Data for every treatment combination of three between-row and four within-row spacing treatments.

[§]Data for between-row spacing treatments are pooled across within-row spacing treatments under each cultivar.

[¶]Data for within-row spacing treatments are pooled across between-row spacing treatments under each cultivar.

number of stems per square meter increased slightly year by year at the 30-, 45-, and 60-cm within-row spacing but had an inverse trend at 15-cm within-row spacing (Table 4, 7).

Racemes per stem were significantly affected by between-row and within-row spacing treatments but responded primarily to increases in within-row spacing (Table 7). The fewest racemes per stem were obtained from the 60-cm between-row spacing treatment over four years. In addition, racemes per stem increased with the increase in within-row spacing except in 2006. There was a year \times between-row spacing interaction for racemes per stem but no clear trend, which indicates an inconsistent between-row spacing effects over years. Furthermore, a decrease in stems per square meter was consistent with an increase in racemes per stem among three between-row spacing and four within-row spacing treatments in 2005 and 2007 (Table 7).

The effects of between-row spacing treatments on pods per raceme, seeds per pod (data not shown), and 1000-seed weight were not significant during the four years. Although the effect of within-row spacing was significant for pods per raceme in 2004 and for 1000-seed weight in 2006 and 2007, no distinguishable trends were detected (Table 7). The heaviest seeds and the least racemes per stem were obtained by WL232HQ in all four years. The differences among cultivars

in stems per square meter, pods per raceme, and seeds per pod were recorded but were not significant over the four years. Of particular interest was that significantly more racemes per stem in 2005 failed to result in increased seed yields, but that can be attributed to weather-related decreases in pods per raceme, seeds per pod, and 1000-seed weight (Table 7).

Plant Height, Basal Stem Diameter, and Lodging Status

There were significant differences in plant height as well as in lodging status among three between-row spacing treatments, with 100-cm between-row spacing having the lowest lodging in 2005, 2006, and 2007 (Table 8).

Compared with other within-row spacing treatments, plants with 15-cm within-row spacing consistently exhibited the shortest height during the three evaluation years (Table 8). No significant differences in plant height were observed among 30-, 45-, and 60-cm within-row spacing treatments. The basal stem diameter increased significantly with within-row spacing increases in 2006. Mean lodging status decreased significantly with each incremental increase in within-row spacing in 2005, 2006, and 2007.

The three cultivars differed significantly in plant height, basal stem diameter, and lodging status. Derby exhibited

Table 7. Average values for stems per square meter, racemes per stem, pods per racemes, and 1000-seed weight under three between-row spacing treatments, four within-row spacing treatments, and three cultivar treatments in 2004, 2005, 2006, and 2007.[†]

	Stems/m ²				Racemes/stem				Pods/raceme				1000-seed weight			
	2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007	2004	2005	2006	2007
— g —																
Between-row spacing treatments [‡]																
60 cm	189a	202a	184a	207a	21.9b	32.3b	18.8b	15.9b	9.73a	6.73a	8.64a	10.4a	1.96a	1.63a	1.83a	1.81a
80 cm	165b	173b	178a	168b	24.5a	35.3ab	20.8a	20.9a	9.64a	6.80a	8.80a	10.8a	1.97a	1.66a	1.82a	1.78a
100 cm	139c	129c	140b	127c	22.6b	38.7a	20.5a	22.9a	9.41a	6.43a	8.73a	11.0a	2.00a	1.66a	1.79a	1.82a
LSD	20	17	16	18	1.8	3.6	1.7	2.4	0.63	0.51	0.66	1.1	0.07	0.03	0.04	0.06
Within-row spacing treatments [§]																
15 cm	237a	225a	211a	204a	19.0c	29.8b	15.7b	17.3b	9.46b	6.70a	8.51a	10.6a	1.94a	1.64a	1.78b	1.76b
30 cm	165b	174b	177b	181b	22.9b	36.1a	20.8a	20.2a	9.21b	6.61a	8.71a	10.7a	2.01a	1.66a	1.81ab	1.76b
45 cm	137c	147c	150c	152c	23.3b	36.9a	20.8a	20.5a	10.31a	6.70a	9.13a	10.6a	1.96a	1.66a	1.83a	1.87a
60 cm	118c	125d	133c	133c	26.8a	39.0a	22.8a	21.5a	9.40b	6.60a	8.54a	10.9a	2.00a	1.65a	1.82ab	1.83ab
LSD	23	20	18	21	2.1	4.1	2.0	2.8	0.73	0.59	0.76	1.2	0.08	0.04	0.04	0.07
Cultivar treatments [¶]																
WL232HQ	157a	169a	166a	161a	22.5b	36.6a	19.8a	19.7a	9.75a	6.79a	8.68a	10.7a	2.07a	1.71a	1.93a	1.90a
Derby	177a	159a	162a	171a	24.7a	35.3a	20.9a	19.8a	9.38a	6.64a	8.96a	10.7a	1.94b	1.61b	1.73c	1.74b
Algonquin	159a	175a	175a	170a	21.8b	34.5a	19.3a	20.2a	9.66a	6.53a	8.52a	10.7a	1.93b	1.63b	1.78b	1.77b
LSD	20	17	16	18	1.8	3.6	1.7	2.4	0.63	0.51	0.66	1.1	0.07	0.03	0.04	0.06

[†]Means in the same column with different letters are significantly different ($P \leq 0.05$).

[‡]Data for between-row spacing treatments are pooled across within-row spacing and cultivar treatments.

[§]Data for within-row spacing treatments are pooled across between-row spacing and cultivar treatments.

[¶]Data for cultivar treatments are pooled across between-row and within-row spacing treatments.

the greatest plant height and basal stem diameter and experienced less lodging than other two cultivars (Table 8).

There were inverse relationships between basal stem diameter and lodging status in 2005, 2006, and 2007, and the correlation coefficients were significant among the three cultivars in 2005 and also significant for Derby in 2006 and 2007 (Table 9). However, correlation analysis did not reveal a consistent relationship between lodging status and plant height, and only Algonquin had a significant inverse correlation coefficient in 2006.

DISCUSSION

Actual Seed Yield

Thinning to reduce plant density has long been known to improve seed yields of alfalfa (Carlson, 1935; Jones and Pomeroy, 1962; Abu-Shakra et al., 1969; Askarian et al., 1995), but the recommended between-row and within-row spacings vary considerably. Askarian et al. (1995) reported that seed yield obtained with 15-cm between-row spacing was significantly lower than those with 30, 45, and 60 cm in the first year and that no significant differences were observed among four between-row spacing treatments in the second year. Furthermore, Rincker (1976) reported that seedlings of alfalfa transplanted 30.5, 61, and 122 cm apart in 91-cm rows produced similar seed yields over a 4-yr period. In our experiment the seed yields using three between-row

and four within-row spacing treatments varied from year to year. In the first year of the study, the highest-density treatments (60-cm between-row spacing and 15-cm within row spacing) produced the highest mean seed yields, primarily because of the higher amount of stems per square meter. In the second year, all three between-row spacing and four within-row spacing treatments had similar mean seed yields with one exception; for Algonquin the 60-cm within-row spacing had a significantly lower seed yield. The response of different yield components varied considerably, however. In the third and fourth years, intermediate density treatments (80-cm between-row spacing and 30-cm within-row spacing) produced the highest mean seed yields. The significant year \times between-row spacing and year \times within-row spacing interactions for seed yield further documented the superiority of the 80-cm between-row and 30-cm within-row spacing treatments as the stand matured. In addition, the combinations of 80-cm between-row and 30-cm within-row spacing optimized seed yields in the third and fourth years in all three cultivars. Our results therefore support the recommendations of Pederson and McAllister (1955) that alfalfa be grown in rows 61 to 91 cm apart with plants spaced about 30 cm apart in the row for seed production. There are two probable reasons for this response. First, plants in intermediate density stands have the room and resources to expand in size by developing more stems, branches, and racemes per stem (Dovrat, 1969 et al.; Taylor

Table 8. Average values for plant height, basal stem diameter and lodging status in three alfalfa cultivars under three between-row and four within-row spacing treatments in 2005, 2006, and 2007.[†]

	Plant height			Diameter at the basal			Lodging status [‡]		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
	cm			mm			Scale (1–5)		
Between-row spacing treatments [§]									
60 cm	167.6a	126.4ab	131.5ab	3.76a	3.63a	3.81b	3.67a	2.93a	3.30a
80 cm	163.6b	123.8b	128.0b	3.79a	3.59a	3.92ab	3.40a	2.82a	3.20a
100 cm	162.0b	127.9a	133.8a	3.86a	3.69a	3.97a	2.94b	2.50b	2.96b
LSD	3.7	3.1	3.9	0.12	0.10	0.13	0.38	0.22	0.16
Within-row spacing treatments									
15 cm	161.7b	122.4b	130.0a	3.58c	3.48c	3.75b	4.14a	3.24a	3.46a
30 cm	166.7a	127.0a	132.0a	3.83b	3.58bc	3.93a	3.42b	2.90b	3.26b
45 cm	164.3ab	125.9ab	131.3a	3.82b	3.67b	3.93a	3.06bc	2.57c	3.01c
60 cm	164.8ab	128.9a	131.2a	4.00a	3.84a	3.99a	2.74c	2.30d	2.88c
LSD	4.3	3.6	4.5	0.13	0.12	0.15	0.44	0.26	0.18
Cultivar treatments [#]									
WL232HQ	156.1c	119.2c	126.8b	3.80b	3.67a	3.86b	3.75a	2.98a	3.40a
Derby	173.2a	134.7a	137.3a	3.93a	3.76a	4.05a	2.92c	2.50b	2.92c
Algonquin	163.9b	124.2b	129.2b	3.69b	3.49b	3.78b	3.35b	2.78a	3.14b
LSD	3.7	3.1	3.9	0.12	0.10	0.13	0.38	0.22	0.16

[†]Means in the same column with different letters are significantly different ($P \leq 0.05$).

[‡]Lodging was scored visually during the flowering period on a scale from 1 (no lodging) to 5 (fully lodged).

[§]Data for between-row spacing treatments are pooled across within-row spacing and cultivar treatments.

^{||}Data for within-row spacing treatments are pooled across between-row spacing and cultivar treatments.

[#]Data for cultivar treatments are pooled across between-row and within-row spacing treatments.

and Marble, 1986), which over time, gradually compensate for low clump density. Furthermore, the probability of flowering usually increases with plant size, thus suggesting an increasing resource availability of individual plants (Snow and Whigham, 1989; Primack and Hall, 1990). Second, high-density treatments such as 60-cm between-row spacing and 15-cm within-row spacing create greater interplant competition, resulting in a negative effect on seed yields (Kowithayakorn and Hill, 1982). Fu et al. (1999) found that with Caucasian clover (*Trifolium ambiguum* Bieb.), seed yields per plant fell significantly as plant density increased. This is probably true for alfalfa also. Brand and Westgate (1909) found that alfalfa plants growing alone produced more seed than crowded plants. In our experiment, the mean seed yields per clump in three cultivars increased significantly with increases in between-row spacing and within-row spacing over the 4-yr period, the only exception being in 2004 when 45-cm within-row spacing treatment gave the highest mean seed yield per clump of Derby. According to Kowithayakorn and Hill (1982), alfalfa seed production depends on seed yield per plant rather than the number of plants per unit area, and wide row spacing promotes more branches, flowers per plant, higher percentage seed set, and higher seed yields per plant. However, there is a point of diminishing returns whereby higher yields per clump cannot entirely compensate for the lower clump density. Seed yields obtained from the combinations of 100-cm between-row spacing and 60-cm within-row spacing consistently had the lowest seed yields in 2005, 2006, and 2007. On the

Table 9. Correlation analysis for lodging status and two morphologic traits (stem height, basal stem diameter) for three cultivars across between-row and within-row spacing treatments in 2005, 2006, and 2007 ($n = 48$).

Cultivars	Plant height			Basal stem diameter		
	2005	2006	2007	2005	2006	2007
WL232HQ	-0.027	-0.189	-0.071	-0.595**	-0.227	-0.168
Derby	0.240	-0.003	-0.149	-0.413**	-0.296*	-0.300*
Algonquin	0.147	-0.356*	-0.125	-0.370**	-0.21	-0.216

*Significant at the $P \leq 0.05$ probability level.

**Significant at the $P \leq 0.01$ probability level.

other hand, the combinations of intermediate seed yield per clump and intermediate clump density under intermediate spacing treatments (80-cm between-row spacing and 30-cm within-row spacing) produced the maximum seed yields in the three cultivars in 2006 and 2007. In conclusion, the key to maximizing yield depends on the optimum balance between clumps per square meter and yield per clump, rather than either of these factors individually. To ensure the establishment of an alfalfa stand, it is advisable to start with an intermediate between-row spacing (80 cm) and higher within-row plant density (15 cm), which can help to maximize the seed yields in the first harvest year. Then, as the stand matures, thinning can be used to decrease the within-row plant density to an intermediate level (30–45 cm). Cross-cultivation can be used to maintain the desired within-row density in maturing alfalfa stands.

Although determining the appropriate agronomic factors that optimize both seed yield and quality is important

(Hampton, 1991; Steiner et al., 1992), we found no significant adverse effects from either low or high plant density on seed germination (data not shown). On the whole, the effects of between-row spacing and within-row spacing on germination were substantially less than that on seed yield. This is probably because plants with a small reproductive load, such as alfalfa, can maintain seed quality to a greater extent than plants with a large reproductive load (Iannucci et al., 2002).

Alfalfa Seed Yield Components

Five seed yield components, especially stems per square meter and racemes per stem, responded differently to the effects of between-row spacing and within-row spacing treatments over the four years of the study.

First, the significant year \times within-row spacing interaction for stems per square meter suggests that from 2004 to 2007, the number of stems per square meter increased slightly with each subsequent year for all but the 15-cm within-row spacing treatments. The decline in stems per square meter with 15-cm within-row spacing may have resulted from enhanced interplant competition for light, water, and nutrients, which eliminated smaller, less vigorous plants and ultimately increased mortality. Racemes per stem was significantly affected by the first incremental increase in between-row and within-row spacing treatments but were not significant among the wider between-row and within-row spacings. Askarian et al. (1995) and Dovrat et al. (1969) stated that increases in racemes per stem with decreasing plant densities can be attributed to the production of more primary, secondary, and tertiary shoots. Our study suggests that the 80-cm between-row spacing and 30-cm within-row spacing are adequate for increasing racemes per stem.

With incremental increases of between-row spacing and within-row spacing came corresponding decreases in stems per square meter, but the thinner stands exhibited increases in racemes per stem. The significant increase in racemes per stem with increasing within-row spacing resulted in comparable seed yields. High seed yields were maintained throughout the third and fourth harvest years (2006 and 2007) despite having lower stems per square meter. These findings are in agreement with other studies that suggest that stems per square meter is not the only deciding factor in determining seed yields but are probably the result of the uninhibited production of racemes per stem in thinner stands (Pederson et al., 1956; Teuber and Brick, 1988). On the other hand, in the later three years, the lowest seed yields were consistently obtained from the combination of 100-cm between-row spacing and 60-cm within-row spacing treatments, suggesting that with the thinner plant density, the increased racemes per stem could no longer compensate for declines in stem per square meter. We can conclude that seed yields are strongly correlated to the combined effects of both stems per square meter and racemes per stem.

Seeds per pod did not significantly change with increases in between-row spacing and within-row spacing over the four years (data not shown). However, these results support the findings of Kowithayakorn and Hill (1982), who concluded that the number of seeds per pod was not an important yield component when the plant density was the primary factor influencing seed yields of alfalfa. The same was found to be true for pods per raceme, as there was no significant response in pods per raceme with increases in between-row and within-row spacing, with no obvious trends detected. Furthermore, 1000-seed weight showed no significant difference among between-row spacing treatments over the four years. In contrast, differences in 1000-seed weight were always significant among the three cultivars over the four years. These data supported the finding of Bolanos-Aguilar et al. (2000, 2002), who reported that seed size in herbage legumes was influenced primarily by genetic factors.

The presentation seed yield presents only a proportion of the potential yield on the standing crop for harvest (Hill and Loch, 1993). The number of seeds produced per unit area multiplied by the average seed weight gives the presentation seed yield in weight per unit area. In this experiment, the presentation seed yields were calculated from the seed yield components, and the ratios of actual seed yields to presentation seed yields were tested, with an average value of about 25% (data not shown). The loss of seed yield mainly came from the processes of harvesting, threshing, and cleaning because these work were done with hand sickles and other hand tools. Thus, the higher seed yields would be realized if more efficient and appropriate machine equipment was used in our experimental conditions.

Effects of Gales

An average of 25 gales was recorded every year in our experimental conditions, which can result in severe lodging. As reported by Bolanos-Aguilar et al. (2002), lodging is unfavorable for seed set because a more compact canopy may limit pollination and possibly induce disease damage to the pods. The threat of damage from gale-force winds is so prevalent that plant growth regulators such as CCC (2-chloroethyltrimethylammonium chloride) have been applied to try to minimize plant lodging, but with limited success (Wang, 2003; Wang, 2005). Furthermore, some plant growth regulators actually had a negative effect on seed production, while others had inconsistent results over years (Wang, 2005). Our studies are in agreement with those found by Pederson (1957, 1962) that lodging status in low-density stands of alfalfa was less and decreased significantly with increasing between-row and within-row spacing. We also found that between-row and within-row spacing treatments had less impact on plant height than on basal stem diameter. The correlation analysis further indicates that the reduction of lodging, as a result of increasing between-row and within-row spacing, was positively

associated with an increased basal stem diameter. Thus, the thicker rather than shorter stems enhanced the lodging resistance of alfalfa plants. To maximize alfalfa seed yield, lodging can be prevented or at least reduced through modifications in between-row and within-row spacing, especially in areas where gales are prevalent.

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